On mice and humans: the role of thymic stromal lymphopoietin in human B-cell development and leukemia

Angela Maria Savino and Shai Izraeli

Leukemia Research Section, Edmond and Lily Safra Children Hospital, Sheba Medical Center, Tel Hashomer, Israel and Department of Human Molecular Genetics and Biochemistry, Sackler Medical School, Tel Aviv University, Israel

E-mail: sizraeli@sheba.health.gov.il *doi:10.3324/haematol.2016.142448*

The development of immunodeficient mouse models has revolutionized the ability to study human disease *in vivo*. One of the difficulties is that many mouse and human cytokines are not cross-reactive. For this reason, the *in vivo* models currently used are not optimized enough to recapitulate the correct environment for every type of human hematopoietic or leukemic cell. This problem has been recently addressed by the development of several models of humanized mice expressing human cytokines, as described in detail by the recent excellent review in *Haematologica* by Theocarides *et al.*[1] In this issue of *Haematologica*, Francis *et al.*[2] created a chimeric mouse able to produce human thymic stromal lymphopoietin (TSLP) to study the effect of human TSLP on human B-cell development and human B-cell precursor acute lymphoblastic leukemias (BCP-ALLs) with abnormal expression of the TSLP receptor (TSLPR) (Figure 1). The generation of this mouse was required since the homology between mouse and human TSLP is only 43% and 35% for the TSLPR, with no cross reactivity between the species.[3]

Thymic stromal lymphopoietin is a cytokine that was first described as a constituent of medium conditioned by thymic stromal cells. TSLP is important in many allergic disorders,

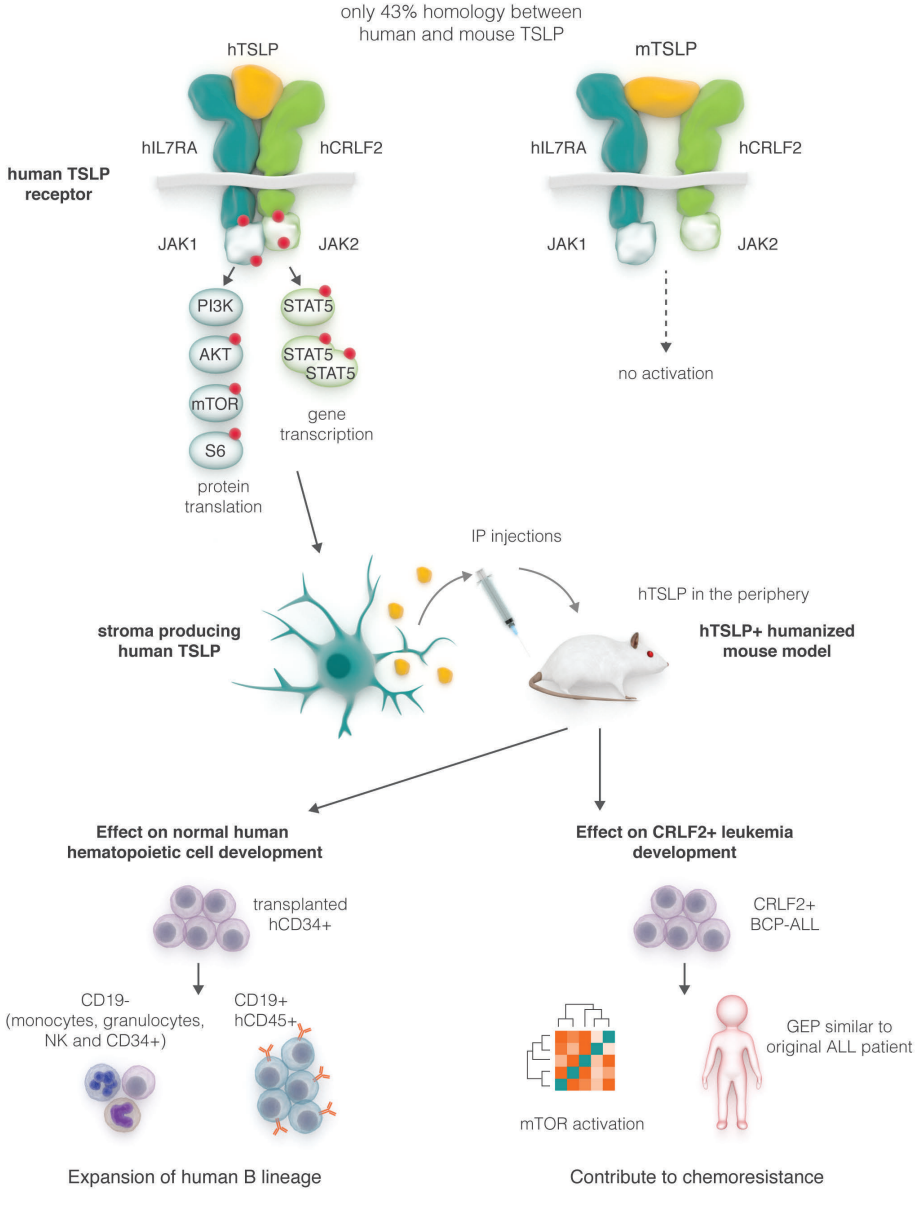

Figure 1. Schematic representation of the humanized mouse model and the effect of human TSLP on normal and malignant hemopoiesis. Only human TSLP is able to induce a conformational change in human heterodimeric receptor and to activate the JAK/STAT or PI3K/mTOR downstream pathway (red dots indicate phosphorylation). The injection of human stromal cells transduced to produce TSLP in NSG mice, induced marked increase of B-cell lymphopoiesis upon transplantation with CD34+ cells derived from human cord blood and, after transplantation with CRLF2 positive leukemia, induced a gene expression similar to the original patient. The increased expression of genes involved in mTOR signaling could be associated with chemoresistance.

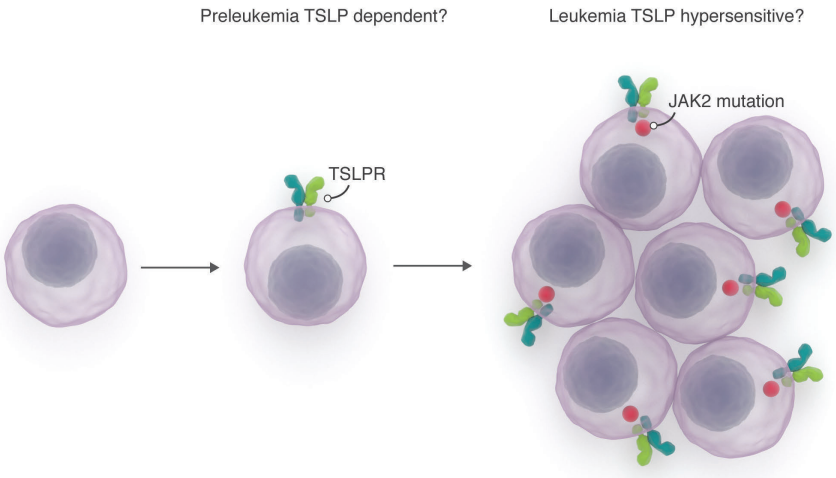

Figure 2. TSLP and CRLF2 positive leukemias: a hypothetical model. In the pre-leukemic phase, B-cell progenitors over-expressing the TSLP receptor are expanded in response to TSLP. In the leukemic phase, acquired activating mutations in the pathway (e.g. in JAK2) make the cells hypersensitive to TSLP, causing their dramatic expansion.

including asthma and atopic dermatitis (reviewed by Tal *et al.*[4]). In addition to the thymic medulla, its major sites of production are the lung, bronchial tree, intestine and the skin.[5] It is currently not known to what degree it is produced by bone marrow stroma, although some studies suggest its expression in some mesenchymal stroma cells,[6] for example, during co-culture with myeloma cells.[7] Its receptor (TSLPR) is a heterodimer composed of one TSLP-binding subunit (CRLF2) and the alpha subunit of interleukin 7 receptor (IL7RA). The TSLPR is expressed on a variety of cells (lymphocytes, mast cells, basophils, monocytes and dendritic cells) and, upon the binding of the ligand, it activates the JAK/STAT signaling pathway.

While TSLP has a role in the development of some subsets of T lymphocytes,[8] its role in mouse B-cell development is unclear. Mice unresponsive to both IL7 and TSLP (lacking IL2RG and CRLF2) have neither B nor T cells. However, in mice lacking only the receptor to IL7, transgenic expression of TSLP partially restored B and T lymphopoiesis, showing a sort of redundancy with IL7.[9] Even less clear is the role, if any, of TSLP in human B-cell development. While some levels of CRLF2 RNA are detected in normal B-cell progenitors, the surface expression of TSLPR is barely detectable. To date, there has only been one *in vitro* study, reporting the co-operation between TSLP and IL7 in the proliferation and differentiation of human fetal B-cell precursors.[10]

The model created by Francis *et al.* allowed the first *in vivo* examination of the role of human TSLP in B-cell development (Figure 1).[2] Human bone marrow stroma cell line (HS27) transduced to secrete human TSLP were given by intra-peritoneal injection to NSG mice and survived in the peritoneal cavity (they were not detectable either in the spleen or bone marrow (BM) of the mice) reaching serum levels of TSLP similar to those of human blood. The advantage of this model consists in the ability to modulate the serum levels of hTSLP based on the timing and number of stromal cells injected. This aspect could be biologically relevant, as underlined by the authors, since the *in vivo* production of TSLP is usually increased by environmental factors. The authors demonstrated that the TSLP produced from the stromal cells was functional, being able to induce *ex vivo* the phosphorylation of STAT5 and p70S6K, which are targets of the two major TSLPR-related pathways.

Importantly, they showed that the presence of human TSLP was associated with a marked increase of human B-cell lymphopoiesis in NSG mice transplanted with $CD34^+$ cells derived from human cord blood. While this observation clearly supports the view that TSLP can affect human B-cell lymphopoiesis, its physiological role remains unclear. NSG mice express mouse IL7 that can only partially stimulate the human IL7 receptor,[11] thus the observed TSLP effect is on the background of a relative lack of IL7. In addition, for reasons that are still unclear, human hematopoiesis in these mice is skewed toward the B-cell lineage. Regardless of these limitations, the findings of Francis *et al.* clearly demonstrate that, despite the almost absent expression of the receptor, TSLP can support human B-cell lymphopoiesis.[2]

Unlike the uncertainty of its importance in normal B-cell development, a clear role has emerged for the TSLP pathway in BCP-ALL. Up to two-thirds of BCP-ALL in children with Down syndrome and 5%-10% of BCP-ALL children and adults without Down syndrome have acquired genomic aberrations leading to a markedly increased expression of CRLF2, and hence the receptor to TSLP, in the malignant blast cells.[12-14] The increased expression is often accompanied by additional somatic activating mutations in the TSLPR pathway, including the CRLF2 or IL7R receptors or the downstream JAK signaling molecules.[15,16] These mutations induce the cytokine independent growth of leukemic cells that nevertheless present extreme hypersensitivity to TSLP.

These genetic events suggest a potential model for leukemogenesis consisting of two stages: initial TSLP dependent expansion of a (pre-leukemic) B cell over-expressing CRLF2, followed by the further acquisition of cytokine independence by activating mutations leading to frank leukemia (Figure 2). While the model suggests that TSLP is essential to the evolution to leukemia, it could also be possible that the extreme sensitivity of the frank leukemic cells to TSLP may protect them from chemotherapy, explaining the bad prognosis of these leukemias.

The model created by Francis *et al.* may be used to study these questions. The authors examined the effect of systemic human TSLP on growth of only two primografts of

human CRLF2 positive BCP-ALL. While they could not observe any effects of human TSLP on the growth of the leukemic cells, their gene expression analysis was more like the original patient sample showing an increased expression of genes involved in mTOR signaling that is known to be associated with chemoresistance.[17] It would have been interesting to see if the presence of TSLP changed the response of these leukemias to chemotherapy.

The new mouse model suffers from two major limitations. It is laborious, requiring weekly injections of a large number of genetically modified stromal cells secreting human TSLP. More importantly, it does not recapitulate the normal sites of production of TSLP and its normal regulation. This could be achieved by either creating an NSG transgenic mouse with human BAC of TSLP or by a "knock-in" of the human TSLP into the mouse TSLP locus. It is important to create such a mouse model, especially in the light of the possibility that TSLP is made by some BM stromal cells.[6,7] Recent studies underline the importance of the human BM niche in providing signals of survival and proliferation of normal and malignant hematopoietic cells (reviewed by Schepers *et al.*[18]). Furthermore, it has already been demonstrated in transgenic mice that the local increase in TSLP production induced systemic alterations in B-cell development.[19] It might, therefore, be reasonable to hypothesize that, in addition to the systemic levels of TSLP, local production of TSLP in the bone marrow may protect residual leukemic cells from chemotherapy. A more physiological humanized TSLP mouse model will be required to test this hypothesis.

The study of the role of human TSLP in BCP-ALL has an important practical significance. If, indeed, CRLF2 positive BCP-ALL depends on TSLP, then drugs targeting TSLP, developed for allergic disorders,[20] may have a role in the treatment of these leukemias.

Acknowledgments

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